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of Dr. S. Waterman

SPECTRUM ANALYSIS OF OLD BLOOD-STAINS.

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S. WATERMAN, M.D.,

NEW YORK.

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REPRINTED FROM

Dr. Brown-Sequard's Archives of Scientific and Practical Medicine.

No. 5, 1873.

NEW YORK:

G. P. PUTNAM'S SONS,

TWENTY-THIRD ST. AND FOURTH AVE.

1873.

VII.

SPECTRUM ANALYSIS OF OLD BLOOD-STAINS.

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S. WATERMAN, M.D.

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THIS paper is directed to the inquiry, whether old dried blood could be spectroscopically demonstrated, and in what manner the spectrum thus obtained differed from the spectrum of blood recently dried. It will be in the recollection of the profession, that this question arose at the trial of Mr. Alley, in Boston, who stood before a jury charged with murder. The State's medical expert stated in his testimony that old blood-spots could not be distinguished from blood-spots of recent date. I intend to prove that we can so distinguish, and that, in this respect, the statement of the expert was at variance with the exact results presented by spectral analysis.

Were I to rely on authority alone, I could quote such well-known physicists as Hoppe-Seyler, Preyer, Gorup-Besonez, Kuehne and others, who, in various publications, have pointed out the spectral appearances of old blood. But I have made extended and patient investigations myself, for a number of years, and had known these varied appearances long before I became acquainted with the views of these writers.

I had two specimens of old blood with which I experimented. One was four years old, and was abstracted by venesection from a female during parturition; the other was solved out from a piece of the blood-stained shirt of the murdered man Nathan, about two years old. In order to ascertain the peculiar optic relations of old blood, it should be brought to a proper state of solution. If the blood is in lumps, it ought to be finely pulverized in a clean mortar, and distilled water added, at a temperature of 60° F. at the highest. A lower temperature is preferable, but it takes longer time to solve the blood, its hæmato-crystallin having lost its solubility to a very great extent by age.

Ten grains of dried blood to an ounce of water gives a proper solution to experiment upon. Having intimately mixed the powdered blood and the water, set the solution aside for 12 hours, at a low temperature, and then filter. Let me here remind those desirous to

investigate, that the more clear the solution is, the more distinct and satisfactory will be the modification of the spectrum. Repeated filtration may become necessary in some cases, especially if the temperature is above 60° F. Your glass tube, or plano-parallel vessel, must be scrupulously clean also. Having made your fluid as clear as possible, submit it to the spectrum test. Note the band in red coincident and even overlapping the C line. (See Spectrum No. 1.) It is never visible when fresh blood is examined, and *is therefore characteristic of old blood.* With the solution concentrated as advised above, you behold this band very well defined; all light absorbed from D to H, with an extension of the red part of the spectrum amounting to several degrees. Having observed this band, dilute your solution 50 per cent. The green part of the spectrum becomes visible, and the two characteristic bands of fresh blood, between D and E, appear dimly outlined, and the rest of the spectrum still overcast beyond the second band at E.

Set your solution aside for another 24 hours, at the common temperature, and then examine again. Look at the remarkable change that has taken place. At first, when only the dark band in red was visible, it seemed as if the blood and its hæmato-crystallin had lost its power to absorb oxygen from the air; it seemed as if by age a complete necrosis or death of the blood had ensued. But now, behold! the two cheerful bands, characteristic of oxyhæmato-crystallin, have reappeared—a resurrection from death. It may need some further dilution to see them well (see Spectrum No. 2); said dilution will cause a corresponding diminution in the distinctness of outline and depth of shade of the line at C, which is, as it were, waning away.

The sign of death yields to the sign of life.

Let your solution stand another 12 or 24 hours in a closed vessel, and on examination spectroscopically mark how the three bands have entirely disappeared, and that between D and E you now behold a dark, broad band, due to deoxidized hæmato-crystallin (see Spectrum No. 3). What has caused this change? Simply your solution has entered a putrefactive process, evolving sulphuretted hydrogen and other gases of decomposition, which in their turn have absorbed or abstracted all the oxygen of the hæmato-crystallin. You have indeed a fair specimen of reduced oxyhæmato-crystallin before you.

To show that this is precisely the case, open now your glass vessel, pour out your solution, and shake it up in some larger glass vessel, with the air of the atmosphere. On submitting the solution again to the spectroscope you will find the broad band of reduced hæmato-crystallin has disappeared, and our old dear friends, the two bands

between D and E, have reappeared (see Spectrum No. 4). They look darker and more defined than ever. Your solution, or the crystallizable material in it, has greedily absorbed and saturated itself with oxygen, showing that, despite long years of death apparent, the hæmato-crystallin has preserved its integrity, and its capacity, when brought again in the proper conditions, to renew its vital mission. The solution at this stage will bear dilution, and will bring out the absorption-bands with rare clearness and depth of shading.

You can repeat the last-mentioned process many a time, and I have not yet ascertained the limit of this absorbing power, although I have satisfied myself that it gradually diminishes.

The next question which arises is, To what changes in the blood is this line coincident with C due?

Is it due to hæmin, which also shows a band in red between B and C?

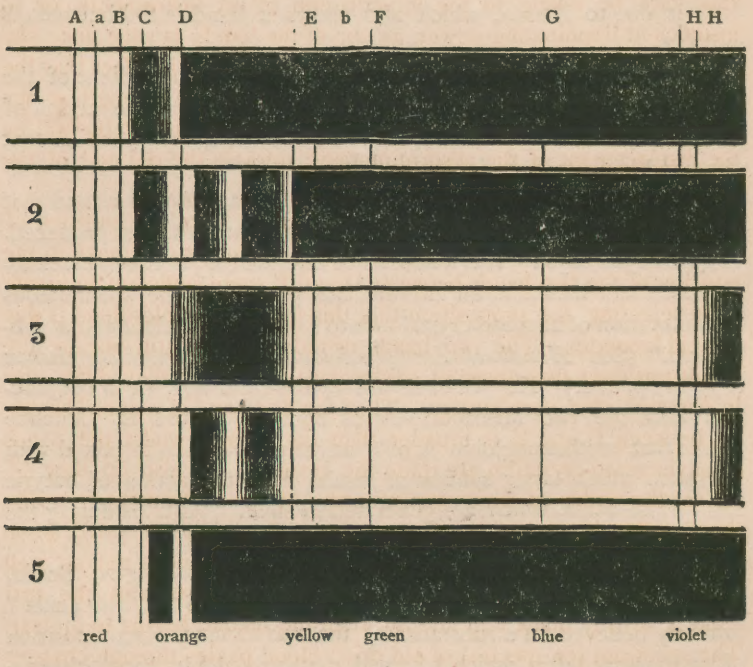
Considering that hæmin is not a physiological ingredient of the blood, but altogether an artificial one, resulting from the action of glacial acetic acid upon blood, we must exclude the proposition that this band is due to hæmin.

With regard to methæmoglobin, it is necessary to understand that no isolated crystalline body bearing that name has yet been produced. Hoppe-Seyler says: "It is a substance intermediate between hæmato-crystallin and hæmatin, an intermediate product of the spontaneous transmutation of hæmato-crystallin into hæmatin and albuminous substances." (See *Handbuch der physiologisch-pathologisch-chemischen Analise*, p. 220.) He places its absorption-band near C, in conjunction with the two absorption-bands between D and E. Kuehne thinks that methæmoglobin is only oxyhæmato-crystallin mixed with hæmatin, which latter substance results from the action of butyric and formic acids, which are generated in small quantities during the decomposition of blood.

Again, in his *Medicinische Chemische Untersuchungen*, Berlin, 1871, p. 378, Hoppe-Seyler says: "In addition to the fatty acids I formerly believed in a substance as the spontaneous decomposition of hæmato-crystallin, which I named methæmoglobin, but further investigation has convinced me, that at the spontaneous decomposition hæmatin and albuminous substances formed at the same time."

Preyer says, *die Blutcrystalle*, p. 196, that methæmoglobin may be said to be a mixture of albumen, hæmatin and iron oxydul in very weak acidulated solution, and when thus viewed, the term methæmoglobin may be considered a superfluous designation. For the present he thinks we ought to retain this designation, however, because of its peculiar absorption-band, and because the solution coagulates.

Under these circumstances, I am not inclined to ascribe the peculiar spectrum to methæmoglobin, because, also, in addition to the foregoing obscurity of what methæmoglobin actually is, the chemical analysis made by me of a solution of dried blood does not correspond with the account given by Hoppe-Seyler, Preyer, Kuehne and others, nor does the position of the band, as given by these authors, correspond with the position, accurately measured by me, which covers and coincides with the line C. It is also of importance to note that when a solution of dried blood is freshly prepared, this line in C is alone visible, and that the two bands due to oxyhæmato-crystallin become only visible subsequently, when the solution has had a chance to absorb oxygen from the air, as shown by the description given above.



But suppose, for argument's sake, that there is such a substance as methæmoglobin. According to Hoppe-Seyler, it is obtained from a solution of hæmato-crystallin, and not from dried blood. See also Preyer, *die Blutcrystalle*, p. 191. I could not verify the results of Hoppe-Seyler that the band at C could be intensified by precipitating solution with acetate of lead (Preyer says a solution of acetate causes no turbidity, nor any precipitation), filtering the precipitation with water, and chymolysis by means of soda-car-

bonate, until all the coloring material is fully dissolved out. On the contrary, I found that after this procedure the band at C had become narrower and more nebulous, and was inferior in depth and breadth to that of the original solution.

Our next inquiry is, then, whether this band is due to hæmatin. Dr. A. H. Gallatin, in his very able paper before the Academy, October 5th, 1871, quoted several authorities to show that this band was due to brown hæmatin. As hæmatin presents a number of spectra, we may well consider first the acid hæmatin spectrum, which, according to Thudicum, shows four absorption-bands, one of which corresponds to our band coincident with the C line. Preyer assigns this beautiful spectrum to hæmatin. I have not been able to discover in the solution of old dried blood more than three bands, but this may be owing to the imperfection of my instruments, or to insufficient illuminating power, as one of the bands is very fine, like a sun-line. Lancaster and others have pointed out the fact that the position of the band in red can be influenced by the strength of the acid employed; the stronger the acid, the more considerable will be the displacement of the absorption-band towards the red end of the spectrum.

Whilst this hæmatin band does not exactly have the position of our band peculiar to old blood, there is still another difference in the position of the two bands between D and E quite sufficient to justify us in rejecting the proposition that this spectrum of old blood is due to acid hæmatin. The two bands of reduced hæmatin occupy different positions than those of oxyhæmato-crystallin; they differ also in size and other peculiarities. Thus the first band of reduced hæmatin between D and E is broader than the second, whilst the bands of oxyhæmato-crystallin are quite the reverse. So also do they differ in the order of their appearance. With oxyhæmato-crystallin the two bands appear simultaneously, and, as already stated, when fully developed, the band near E is the broadest, but is not so well defined as its narrower mate. With reduced hæmatin the first band is already black and intense when the second begins to appear. The spectrum (or spectra) of old dried blood passes through changes very peculiar, and not in any way resembling those of hæmatin.

If hæmatin is at all present in dried old blood it is difficult to understand why the oxyhæmato-crystallin bands and not the hæmatin bands should prevail, and why the broad band of reduced hæmato-crystallin should finally ensue, and not the two bands of reduced hæmatin, and how out of this broad band the two oxyhæmato-crystallin bands should reappear, showing the latter in its full vitalized integrity.

This band in red is probably due to the action of the various gases which are evolved during the process of drying, and which betray their evanescent character when such dried blood is solved in water and exposed to the verifying effects of oxygen.

Hoppe-Seyler describes a spectrum of blood when acted upon by sulphuretted hydrogen. It gives the two normal blood-bands and a third in red near C (see Spectrum No. 5); the absorption is increased of all the blue and green part of the spectrum, so that all color is cut off beyond the third band at E (see Spectrum No. 5).

It seems to me this spectrum answers best to that of old dried blood, and until we learn more of the laws and optical relations of putrefactive processes we may accept it as a provisional explanation of the spectral phenomena above described.

A chemical test for old blood-stains is communicated by Gorup-Besanez, *Anleitung zur Zoochemischen Analyse*, on the authority of Pfaff, who recommends to use a solution of arsenious acid, 1 part to 120 of water. Fresh blood-spots are solved in a few minutes; blood of one or two days old requires 15 minutes; of four or six months, 3 to 4 hours; and, when nine year old, 4 to 8 hours are required.

ARCHIVES

OF

Scientific and Practical Medicine.

This Journal, edited by Dr. BROWN-SEQUARD, with the assistance of Dr. E. C. SEGUIN, and of several New York, Boston, Philadelphia, and Cincinnati Physicians and Surgeons—will appear on the 15th of every month, in numbers of about one hundred pages, similar to the first one.

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Subscriptions received by Messrs. G. P. Putnam's Sons, Association Building, Fourth Avenue and 23d Street, New York City.

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